Resistance to Thyroid Hormone: Probable De Novo Mutation (P453S) in the Receptor Beta Gene

Abstract
Resistence to thyroid hormone (RTH) is a syndrome characterized by decreased responsiveness of target tissues to the action of thyroid hormone (TH), usually due to mutations in the thyroid hormone receptor (TR) β gene. We studied a Turkish family whose proband, a 19-year-old male, presented with diffuse goiter, nervousness, and palpitation. Thyroid function tests revealed elevated levels of TH and nonsuppressed thyrotropin (TSH). Gene sequencing revealed a mutation in one allele of the TRβ gene in the proband, his two brothers, and father. It involved the substitution of the normal cytosine 1642 with a thymidine, resulting in the replacement of the normal proline 453 with a serine (P453S) in the T3-binding domain of the TRβ which is known to have one quarter to one third the T3-binding affinity of the normal TRβ. Genetic study of the family suggests that the mutation may have occurred de-novo in the father of the proband. Turk Jem 2009; 13: 43-6

Key words: Resistance to thyroid hormone, thyroid hormone receptor beta, mutation

Introduction
Resistance to thyroid hormone (RTH) is a syndrome characterized by decreased responsiveness of target tissues to the action of thyroid hormone (TH) (1). The common features of RTH include elevated serum TH and normal or slightly increased thyrotropin (TSH) concentrations. The clinical presentation of RTH is variable. The majority of individuals are completely asymptomatic. Some may manifest symptoms suggestive of TH deprivation such as growth retardation, impaired cognitive ability, and learning disabilities, while others show signs of TH excess such as advanced bone age or hyperactivity and tachycardia (1,2). The first case of RTH was reported by Refetoff in 1967 (3). Mosaicism of the TRβ gene was described for the first time in a Turkish patient by Mamanasiri as a cause of variable sensitivity to TH in different tissues (4).

RTH is generally transmitted in an autosomal dominant manner, but sporadic, de novo cases are also common (5). RTH is mostly caused by mutations in the thyroid hormone receptor (TR) β gene (6). The mutant TR molecules have either reduced affinity for triiodothyronine (T3) or impaired interaction with one of the...
Materials and Methods

Patients

The proband was a 19-year-old male who presented with diffuse goiter, nervousness, and palpitations that have persisted for approximately 5 years. Treatment with propylthiouracil (PTU) was initiated 3 years earlier, assuming that he had a toxic diffuse goiter. He was admitted to the Endocrinology Unit of Suleyman Demirel University with the above mentioned complaints after having ceased the PTU treatment. He weighed 50 kg, his height was 172 cm, blood pressure was 120/80 mmHg, and pulse rate was 96 beats/min. He had a moderate, diffuse goiter and anxious mood. Physical examination was otherwise normal. Laboratory evaluation revealed normal blood count, renal and hepatic function tests and urinalysis.

Thyroid function tests showed a TSH of 1.79 mU/L (normal range 0.4-4.0), a free thyroxine (T4) of 4.4 ng/dl (normal range 0.8-1.8), and a T3 of 5.6 pg/ml (normal range 1.6-4.6). Magnetic resonance imaging revealed a normal pituitary gland. Administration of L-T3 did not result in the usual TSH suppression. Thyroid function tests of first-degree relatives demonstrated that his father, sister and two brothers had elevated serum TH levels with normal TSH concentrations. The patient and 21 family members underwent laboratory and genetic analysis.

Laboratory analyses

Total T4, total T3, and TSH were measured by chemiluminescence using Elecsys 2010 technology (Roche Molecular Biochemicals GmbH and Hitachi, Ltd., both located in Indianapolis, IN). Total reverse T3 and thyroglobulin (TG) were measured by RIA. FT4 index was estimated by calculating the ratio of T3 resin uptake and the total T4 concentration. Thyroid peroxidase and TG antibodies were measured by an agglutination method. Genomic DNA was extracted from peripheral blood leukocytes of the proband and 21 family members. Genomic DNA was amplified by the polymerase chain reaction and all coding exons and intron junctions of the TRβ gene were sequenced using oligonucleotide primers, described previously (12). A written informed consent was obtained from all subjects for genetic analysis and the study was approved by the local ethics committee. The study was also approved by the Institutional Review Board of the University of Chicago.

Results

We identified the same mutation in one allele of the TRβ gene in the proband (subject III-3 in Figure 1), his two brothers (III-5 and III-6) and father (II-8). It involves the substitution of the normal cytosine 1642 with a thymidine. This results in the replacement of the normal proline 453 with a serine (P453S) in the T3-binding domain of the TRβ. All four subjects harbouring the mutation exhibited the typical phenotype of RTH including increase in the concentration of all iodothyronines (T4, T3 and rT3), normal or slightly elevated TSH and, with the exception of subject III-5, high serum TG concentration. The mild increase in FT4 and rT3 concentrations of subject II-5 is compatible with the use of L-T4. We have no explanation for the slight increase of rT3 in subjects II-7 and III-1, and for the slight reduction of TSH in subject II-3. Four individuals had autoimmune thyroid disease based on the detection of thyroperoxidase antibodies (see Fig. 1). None of the 6 sibling of the affected father (II-1-7), nor the paternal grandmother (I-1), or 8 nephews and nieces of the deceased paternal grandmother of the proband (II-10-17) expressed the RTH phenotype. Thus, it is likely that the father has a de novo TRβ gene mutation.

Figure 1. Pedigree of family Mdn and results of thyroid function tests and genotyping. Circles are females and squares indicate males. A line through the symbol indicates that the subject is deceased. The proband is indicated by an arrow. Generations are indicated by Roman numerals and subjects by Arabic numbers to the right of each symbol. Results of thyroid function tests are aligned below each symbol. Abnormal values are in bold numbers. Half shaded symbols indicate the presence of the P453S mutations in one allele of the TR gene. Note that the sister of the proband was not tested due to loss of her blood sample during shipping.
Discussion

RTH is characterized by elevated serum levels of TH and normal or slightly increased serum TSH concentration that responds to TRH (17). Although the precise incidence of RTH is unknown, a limited neonatal survey estimated an incidence of 1 case per 40,000-50,000 live births (18). While most thyroid diseases show a female preponderance, RTH occurs in males and females with equal frequency (17). Familial occurrence of RTH has been documented in approximately 75% of cases. Inheritance is autosomal dominant, with the exception of the RTH caused by complete deletion of the protein-coding of the TRβ gene, which had autosomal recessive inheritance (19).

Before TR gene defects were recognized, patients with RTH were clinically classified as having generalized resistance to TH (GRTH) and selective pituitary resistance to TH (PRTH), based principally on symptoms and signs. Those with GRTH appeared to be eumetabolic and those with PRTH, hypermetabolic, based on restlessness and tachycardia. However, it has been suggested that PRTH may not constitute an entity distinct from GRTH (20). In fact, the clinical manifestations are variable among the families with RTH and also among the affected family members. Furthermore, one and the same subject with RTH may exhibit symptoms and signs of hypothyroidism in one tissue, while in another tissue, findings may be suggestive of thyrotoxicosis. This situation results from the dissimilar distribution of TR isoforms among tissues. For example, TR is expressed predominantly in the heart, bone and brain, whereas TRβ is more abundant in the liver and kidney (22).

At least three different molecular alterations may cause reduced sensitivity to TH in humans: (a) mutations in the gene encoding TRβ isoform causing RTH, (b) mutations of the specific TH transporter, monocarboxylate transporter 8 (MCT8), and (c) mutations in selenocysteine insertion sequence binding protein 2 (SECISBP2) which reduces the synthesis of selenoproteins, including the TH deiodinases (22).

Approximately 85% of subjects with RTH, studied at the gene level, have mutations in the TRβ gene, located on chromosome 3. The TRβ molecule contains a DNA-binding domain, a hinge region, and a ligand (T3)-binding domain. Mutations are located in the T3-binding domain and its adjacent hinge region. Most are single amino acid substitutions with fewer single amino acid insertions or deletions and even less large deletions. Almost all mutations cluster around the ligand-binding pocket observed in the ligand-binding domain of the TRβ crystal structure (1,23). 122 different mutations have been so far identified in approximately 300 families (10). We identified a mutation in one allele of the TRβ gene of the proband, his two brothers and father. The single nucleotide substitution resulted in the replacement of the normal proline 453 with a serine (P453S) in the T3-binding domain of the TRβ. This identical mutation was reported in 9 other families (11-16 and personal observations), 16 families-P453T (12, 16, 29-36 and personal observations), 1 family-P453N (personal observation), 1 family-P453Y (16), and 3 families-P453H (11,31,37). RTH is rarely diagnosed at birth as neonatal screening programs are most often based on the determination of TSH concentration in dried blood. Screening programs that measure both TSH and T4, and using an assay reliable in the high T4 range, would rarely identify a case at birth. However, children of women with known TRβ gene mutations should be tested either prenatally or at birth.

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